Physiological Monitoring in Diving Mammals

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LONG-TERM GOALS

The objective with this study was to develop and calibrate an invasive data logger to measure muscle O_2 saturation in large, freely diving whales. We wanted to study physiological responses during diving in free-ranging, deep diving cetaceans. The idea was to measure muscle O_2 saturation and determine how blood flow to muscle is altered during diving. These data will be important to determine if muscle

blood flow is reduced during diving, and important to estimate how the dive response affects muscle N_2 levels and the risk of decompression sickness (DCS).

OBJECTIVES

This project is separated into three aims:

Aim 1: Develop a new generation of tags/data logger for marine mammals that will contain a sensor to be implanted into the muscle. The logger will collect physiological data from muscle tissue in freely diving marine mammals. The sensor will be tested and calibrated in terrestrial mammals at Massachusetts General Hospital, Boston.

Aim 2: The data logger will be tested in freely diving marine mammals in the field, and muscle O_2 saturation data will be collected.

Aim 3: Measure the concentration of aerobic/anaerobic enzymes, total myoglobin, and fiber type in muscle tissues of post-mortem stranded whales.

APPROACH

This project is separated into three aims: Aim 1a) Development of a new generation of tag/data logger for marine mammals that will contain a sensor to be implanted into the muscle. The logger will collect physiological data from muscle tissue in freely diving marine mammals. The sensor will be tested and calibrated in terrestrial mammals at Massachussetts General Hospital, Boston. The calibration in a terrestrial mammal model is based on the assumption that the absorption spectra is similar for all mammals, which was tested in this fiscal year; Aim 2) The data logger will be tested in freely diving marine mammals in the field, and muscle O_2 saturation data will be collected; Aim 3) The aerobic and anaerobic enzyme and myglobin levels in the muscle of selected species will be measured. The latter is important to convert O_2 saturation to O_2 content.

Aim 1: For aim 1, signifciant progress has been made to develop a more accurate method to esimtate muscle O_2 saturation by the Zapol group (see below)

Aim 2: We completed one translocation of elephant seals in 2013, and the data were analyzed and reported in a thesis (see below)

Aim 3: Two publications were submitted and accepted for aim 3. See below.

WORK COMPLETED

Aim 1:
Introduction
Last September the sensor had been proved to work on Intralipid plus India ink phantoms. However, several aspects of the sensor were pending, notably tweaks that would transition the device from laboratory only instrument to a field usable instrument. It was also pending to implement the simultaneous acquisition of two wavelength measurement, a vital part of the sensor.

Most of the work performed on the project this year was focused on reducing the footprint of the device, with the main objective of reducing its size, cost and energy consumption, while increasing its portability and mechanical stability. Most of those goals were met and now the device is operating while having a reduced size and cost.

Improvements

The work this year consisted on the following six aspects:

1. Implementation of an oscillator
2. Implementation of the second wavelength
3. Simplification of the optics
4. Implementation of the data storage
5. Optimization of the detector fiber
6. Optimization of the phase detector

In Figure 1 we show the current block diagram after all the changes, representing the first complete prototype version. In the following sections we will describe the changes with respect to the previous version.

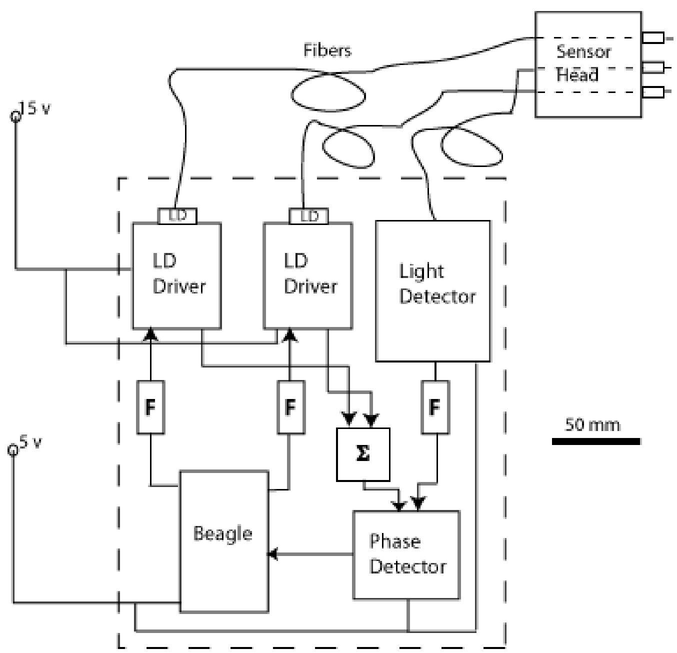

Figure 1: Current diagram of the sensor. The blocks are drawn to scale (power supplies, cables and connectors simplified)

Implementation of an oscillator

The principle of operation of the sensor requires modulating laser light to a frequency in the order of the radiofrequencies (RF). This sensor in particular was designed to work at 70 MHz. Previously, the laser was modulated using a bench top function generator, a versatile device that is nonetheless bulky and designed for laboratory use and requires a 127 V power supply. Eliminating the need of a function generator was thus the final necessary step to create a portable device.

The oscillator was implemented using a microprocessor (BeagleBone Black), as proposed last year but only recently implemented. The device has several advantages, including ease of programming, low cost, size and energy consumption as well as being a multipurpose device. The last point is relevant because it means we can increment the complexity and functions of the system without increasing its size.

The actual implementation ended up being not so straightforward. While the BeagleBone Black has a 1 GHz microprocessor, its analog outputs are PWM with a max frequency of 50 MHz. In order to obtain the required 70 MHz sine wave, we instead created a 10 MHz rectangle pulse wave and used a 70 MHz band pass filter (KR electronics 2657 sma) to filter out everything but the 7th harmonic. The approach worked, but with the caveat that the amplitude of the sine wave is limited to five fixed values adjusted by changing the duty cycle. It could be desirable to change the amplitude to tweak the system further, which would require implementing a dedicated crystal oscillator. Figure 2 shows the resulting sine wave as measured by an oscilloscope. Figure 3 compares the typical size of the signal generator with the BeagleBone microprocessor.

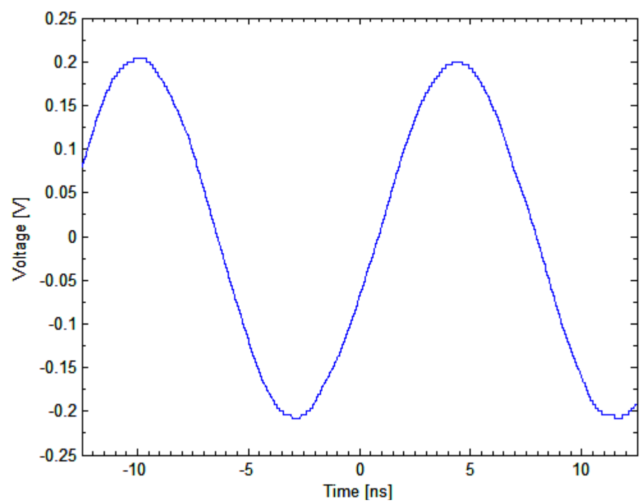

Figure 2: Oscilloscope plot of the 70 MHz sine wave generated using a PWM output of the BeagleBone plus 7th harmonic filtering, eliminating the need for a signal generator

Figure 3: Beaglebone black (bottom left) compared to signal generator. Note the difference in size.

Implementation of the second wavelength
In order to operate, the sensor needs to measure the reflected light of two different wavelengths. The implementation of a second wavelength is a vital aspect of the sensor that was not completely solved.

Our approach was using two different laser diodes, each driven with a different oscillator. Both oscillators were implemented with the Beagle Bone as described in section 1.2.1. In order to use a single detector, we turn the oscillators on and off in an alternating manner, such that only one is on at a given time. This way, the detector will measure only a single wavelength at a time. The alternating logic was implemented with the BeagleBone.

Currently, the system is configured to make a measurement every 2 seconds. This is meant to simplify the experiments and testing, but it could be made faster in the future depending on the goals and test results.

The sensor also needs a reference signal in order to calculate the phase shift in the sample. The reference was implemented by summing the reference output of both LD drivers with a power combiner (Mini circuits ZFRSC-42-S+).

Simplification of the optics
In the previous version of the device, the light collected from the sample traveled through an optic fiber, and then was collected with an optical system to the detector. The optical system was bulky (similar to the size of the current sensor), cumbersome and susceptible to misalignment. We devised a mount to couple the detection fiber directly to the detector, increasing the mechanical stability of the system, greatly reducing the possibility of misalignment and the size and weight of the system.

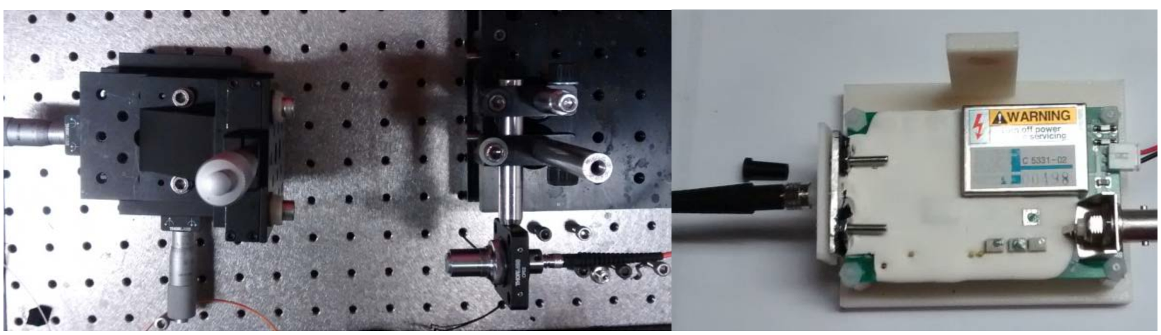

Figure 4: Some of the components of the original optics (left) vs the new (right). Notice the fiber on both figures for a comparison of size

Implementation of the data storage
Up until now the readings of the sensor had been visualized with an oscilloscope. One of the requirements for field implementation is for the sensor to be able to store the samples. We used the BeagleBone data storage function in order to add data recording functionality to our sensor. We are recording the phase detector output using the analog inputs of the BeagleBone Black (12 bit digitizer, meaning a resolution of 440 microvolt). The storage is synchronized with the alternation of the driver signals (described in section 1.2.3) in order to know which of the wavelengths is being measured at a given time. We added a timestamp to the data so it is more easily analyzed.

The signal is stored once per measurement. This is done to maximize the storage space of the microprocessor.

Optimization of the detector fiber

The detection of the light signal is done through an optic fiber in order to reduce the invasiveness of the sensor. The tradeoff is the amount of light collected. Signals are expected to be low, so it is important to maximize light collection to have the best signal to noise ratio possible. We tried several different fibers with different numerical apertures (NAs) and core diameters. No further gain was achieved in our tests with fibers having a core diameter higher than 200 microns and NA of 0.22. It should be mentioned that a higher diameter makes the fiber easier to snap accidentally. We also implemented a micro lens at the tip of the detector fiber in order to increase the amount of collected light (see Figure 5). It modestly improves the signal to noise ratio of the detected signal.

Figure 5: Detection optic fiber with micro lens fitted on the tip (left: picture, right, micrograph).

Optimization of the Phase detector

The previous year we implemented a solid state phase detector in the system, eliminating the necessity of a network analyzer for the sensor. This year we tested different configurations of the phase detector trying to optimize sensitivity versus dynamic range. While the signal to noise ratio of the system benefits from higher sensitivity, we determined that we need the maximum dynamic range in order for the sensor to function.

Aim 2:

A MSc thesis (Measurements of O_2 saturation in freely deep diving Nothern elephant seals *Mirounga angustirostris* using a novel data logging tag, 2015, Texas A&M University – Corpus Christi). From the thesis, one paper on the absorbance spectra of human and marine mammal hemoglobin has been submitted and is under review.

Aim 3:

This aim was completed last year and published in a PhD thesis (Diving physiology in marine mammals: significant findings in pinniped muscle physiology and trachea morphology, https://beardocs.baylor.edu/xmlui/handle/2104/457/browse?value=Moore%2C+Colby+D.&type=author), and two peer reviewed papers.

Moore, C., Crocker, D. E., Fahlman, A., Moore, M., Willoughby, D. S., Robbins, K., Kanatous, S. B. and Trumble, S. J. (2014). Ontogenetic changes in skeletal muscle fiber type, fiber diameter and myoglobin concentration in the Northern elephant seal (Mirounga angustirostris). *Frontiers in Physiology* 5.

Moore, C. D., Fahlman, A., Crocker, D. E., Robbins, K. A. and S.J., T. (2015). The degradation of proteins in pinniped skeletal muscle: viability of post-mortem tissue in physiological research. *Conservation Physiology* 3, 1-8.

RESULTS

Aim 1:

Tests

We performed a test experiment of the first prototype on cuvettes filled with Intralipid and varying concentrations of India ink. We show the results on Figure 6.

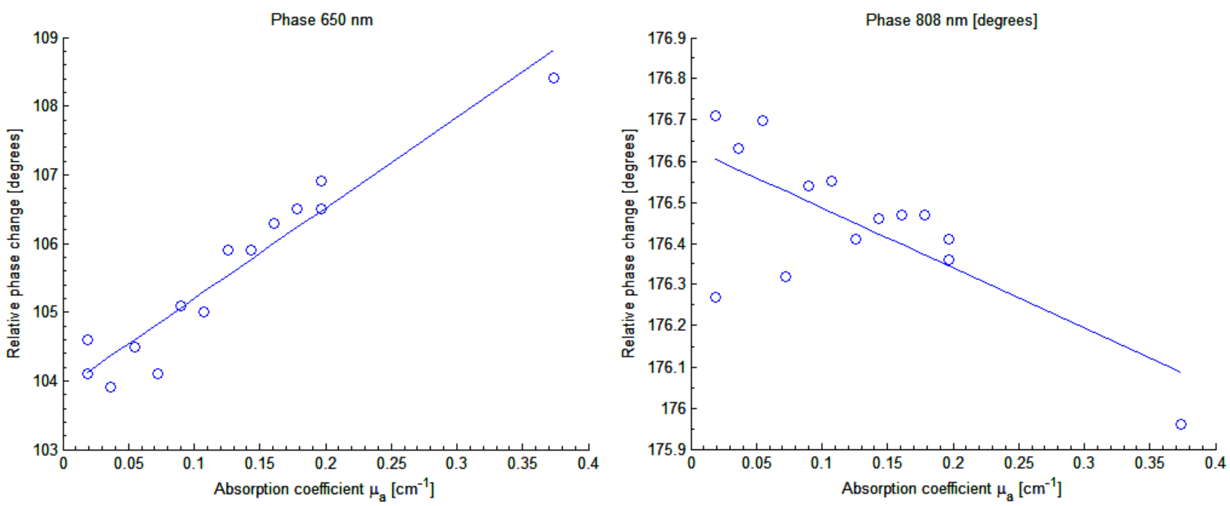

Figure 6: Phase change as a function of absorption coefficient on an Intralipid 1.25% phantom plus India ink. The horizontal axis assumes absorption coefficient of India ink is wavelength independent.

We can clearly notice a correlation between the relative phase change and the absorption coefficient ($p \ll 0.05$). The phase detector is a fairly linear device except near 180°, potentially explaining why the phase at 808 nm deviates the most from the line fit. The electric noise also contributes to the experimental points deviating from a line. Nevertheless, the phase detector is successfully measuring changes in the absorption coefficient, which in living tissue are mainly caused (at those wavelengths) by changes in blood oxygen saturation.

Current status - Milestones

Progress was made on this device during this year

- The first portable prototype version was finalized

- The system is currently working (in Intralipid phantoms with similar optical properties to human tissue) without depending on external bulky devices
- By eliminating the external devices, we managed a size reduction of about 80% (not counting power supplies)

Unsolved issues
There are still some problems that need to be solved before having this prototype make the jump from a lab to a field application.

Energy consumption
While most of the components of the prototype use low voltage (5V) and current, the laser drivers require 15 V and a moderate current, equaling a power consumption of 10 W for both of them. While this is not a concern in a laboratory setting, it means they would quickly discharge a small battery. Several strategies could be pursued in the future, including changing the bias current of the drivers or having the system go to sleep every certain number of measurements and waking up after several hours or until something happens (maybe a prompt by a pressure sensor).

Phase shift
Due to several practical reasons including the length of fibers and cables, there is a hard to characterize offset between the measured phase shift and the actual phase shift caused by the sample. The phase shift will be proportional to changes in oxygenation on the sample, but in order to obtain the actual oxygenation value it is necessary to post process the recorded phase shifts. We still need to determine the processing algorithm to achieve that. While it could be done in-sensor, we believe it is better to do it in a computer after recovering the probe, in order to save energy and recording speed.

Field model casing
The system would need to be encased in a waterproof case with good thermal dissipation.

Clock
The system's clock gets reset every time the BeagleBone is turned off. This is a minor issue, but combined with 4.2.2 and 4.2.1 it could complicate the interpretation of the recorded data.

Things left to do
Currently, we are working in two lines of action: the casing of the device, and tests on a more accurate model than a phantom.

Casing
We are working on designing a case for the electronic components. The objective is having everything inside a single box, making a portable system while protecting the components. This will be a prototype case rather than a final case, meaning it will not be water resistant and the power supplies will be external. The case will have output terminals to be able to monitor several internal signals while performing test experiments, but those will not be present on the final version.

Animal Model
So far, most tests have been performed on an Intralipid phantom. A test experiment was performed with cuvettes full of pig blood with different levels of oxygenation. However, the test was unsuccessful due to the high absorption of pure blood, which rendered the signal to low to be measured. Real tissue

has a blood volume of 0.1 to 10%, so we expect a higher readable signal from a more accurate animal model. Signal is readable on human skin. We are planning on performing tests on dead rats before testing in vivo on a sheep.

Aim 2:

Sensor Implantation and Data Logger Attachment

In April of 2013, elephant seal translocation experiments were conducted to test the ability of a novel sensor and data logger to collect physiological data *in vivo* in deep diving northern elephant seals. Five animals (Table 1) were sedated on the beach and transported to Long Marine Laboratory where the data logger was attached and the oximeter sensor implanted under injectable sedation. In addition, the implantation procedure of the oximeter was assessed and we carefully noted any indications of trauma or changes in swimming ability to the animal. Woven wires attached the internal sensor and external data logger, which allowed flexibility and movement and prevented sheering of the muscle/blubber interface.

Once the sensor and cables were implanted, the cable was secured to the skin at the site where it exited adjacent to the muscle. The data logger was mounted externally using 5 min epoxy (Loctite). The animal recovered overnight and the animal's ability to move and swim assessed. The next morning the seal was transported to Monterey Bay, California, where it was released. The animal swam back to the rookery providing us with physiological diving data stored in the data logger. Once back on the beach, the animal was recaptured and the sensor and data logger removed.

Post-surgical observations (inflammation, infection, and swim behavior)

Findings from implantation resulted in relatively little inflammation and no signs of infection in all 5 of the implanted seals. In all of the seals, muscle placement of the sensor was confirmed and in no case was the peritoneum compromised. There were no changes observed in swim behavior from pre-surgery to post-surgical implantation of the sensor and attachment of the data logger. Following removal of the tags, the site incision/removal site was observed for any trauma. In no seal did we observe any signs of infection, or inflammation.

Findings from the sensor and data logger

Return to the rookery for all translocated seals took 2-7days. GPS tracks for all animals are in Figure 7. Dive profiles for both seals with oximeter data are summarized in Table 2. Data recovered from the data logger of G5944 resulted in little over 3 hours of diving and with no data from the photosensor (Figure 8, Table 2). Eighteen hours of data were recovered from G5882 and the dive record looked very similar during the first 3 hours as compared with G5944 (Figure. 8 vs Figure 9, Table 2). In G5882 changes in LED absorption occurred when G5882 began diving > 350 m approximately 12 hours after the seal was released. Oximetry data was collected for 14 dives out of a total of 77 dives recorded.

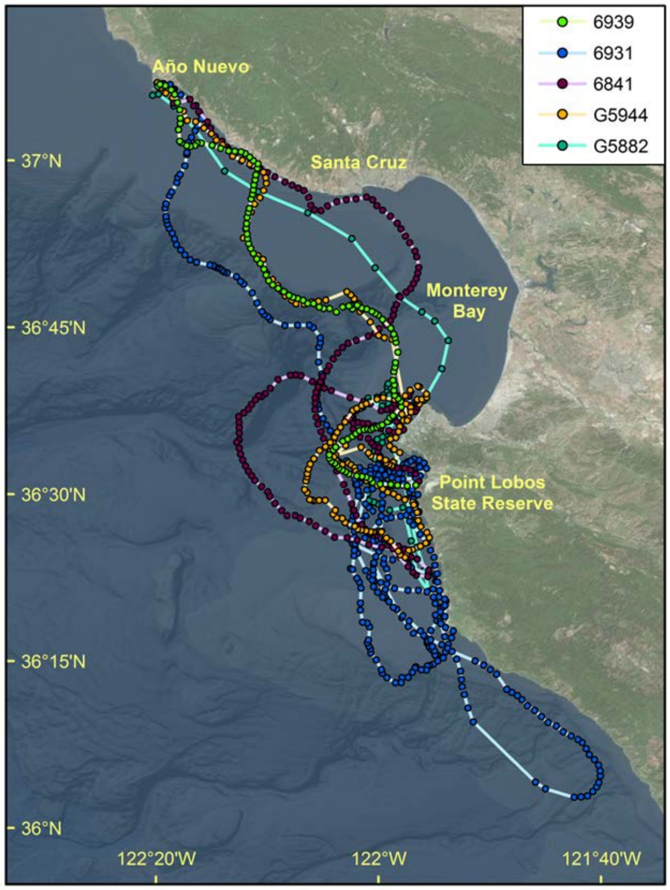

Figure 7. GPS tracks of all 5 Northern elephant seals deployed with oximeter logger. Seals 6939, 6931, and 6841 deployed with the logger implanted, but had no open ocean oximeter data logged. Seals G5944 and G5882 with open ocean data logged. The GPS tracks of all 5 animals overlaid illustrates all the animals swam around the same areas. Note 6931 came back on shore a number of times prior to swimming back across Monterey Bay.

Table 1. Data retrieved from oximeter data logger. Dive profiles recorded for both seals (G5944 and G5882) equipped with the oxygen sensor and data logger and then translocated across Monterey Bay, California.

Seal ID	**Dive Record**				**Dive depth (m)**		**Dive duration (min:sec)**		**Surface Interval (min:sec)**	
	No. hrs recorded	**No. of dives**	**No. of dives/hr**	**% time at surface**	**Max**	**Mean ± SD**	**Max**	**Mean ± SD**	**Max**	**Mean ± SD**
G5944	3:06:58	24	8	14.1	94.53	48.1 ± 32.3	13:14	6:42 ±3:06	8:35	1:06 ± 1:39
G5882	18:17:37	77	4.2	12.2	419.7	146.1 ± 141.0	25:00	12:31 ± 6:48	5:00	1:45 ±0:46

Table 1. The dive profiles of all seals from the GPS loggers with ARGOS satellite uplink once translocated to Pt. Lobos, CA as they swam back across Monterey Bay, CA to Año Nuevo, CA. Mean descent (D) rate, mean ascent (A) rate, mean and max surface interval (SI) are included.

Seal ID	**# Days**	**# Dives**	**Mean dive depth ± SE (m)**	**Max depth (m)**	**Mean dive duration ± SE (s)**	**Mean D rate ± SE (m/s)**	**Mean A rate ± SE (m/s)**	**Mean SI ± SE (s)**	**Max SI (s)**	**Dives /day**
6931	8	601	200 ± 153	711	866 ± 355	0.90 ± .018	0.83 ± 0.20	106 ± 201	3742	75
6939	3	154	158 ± 123	494	964 ± 352	0.66 ± 0.12	0.73 ± 0.15	143 ± 373	4654	51
6841	4	282	181 ± 167	566	795 ± 417	0.75 ± 0.21	0.86 ± 0.28	185 ± 489	4816	71
G5944	3	196	175 ± 137	457	836 ± 405	0.76 ± 0.17	0.80 ± 0.18	100 ± 102	1254	65
G5882	6	472	191 ± 170	681	883 ± 411	0.78 ± 0.21	0.76 ± 0.24	150 ± 373	4890	79

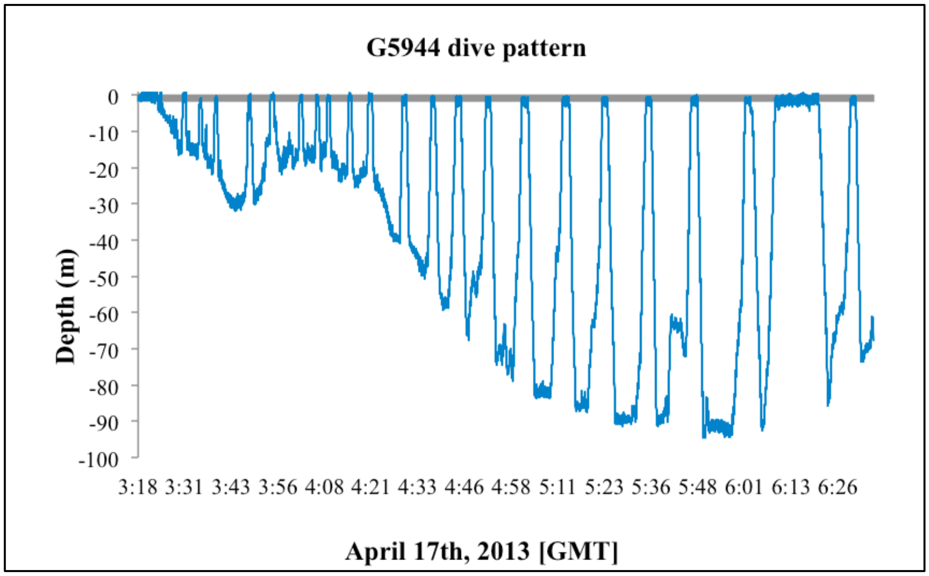

Figure 8. Dive depth (meters) and duration (minutes and seconds) recorded with oximeter data logger for seal G5944 for ~ 3 hrs on April 17, 2013, when translocated across Monterey Bay, California.

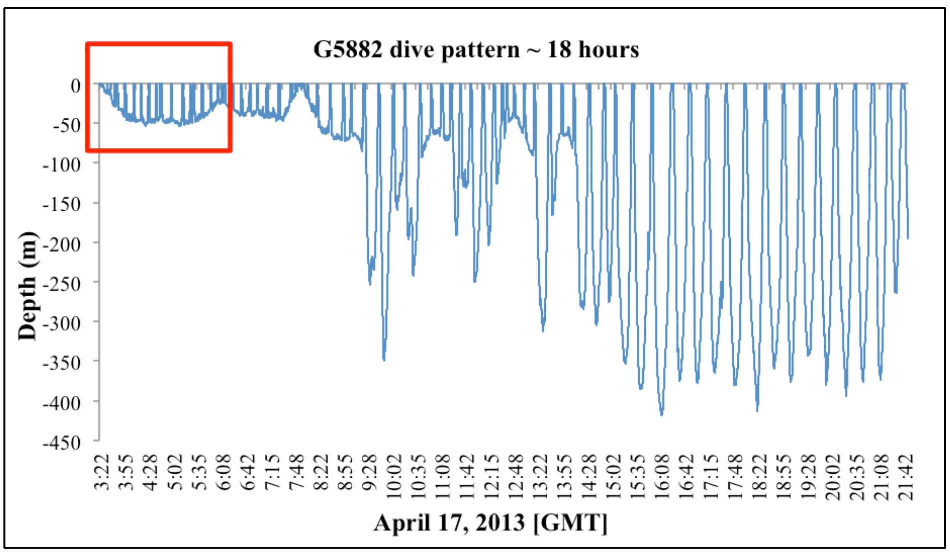

Figure 9. Dive depth (meters) and duration (minutes and seconds) recorded with oximeter data logger for seal G5882 for entire ~18 hours recorded on April 17, 2013 when translocated across Monterey Bay, California. The red rectangle highlights the first 3 hours for comparison to G5944 in Figure 8.

The profile for G5944 with 3 hours of data had a mean depth of 48.1 ± 32.3 m for a total of 24 dives (Table 6, Figure 8). In the 3 hrs of diving, G5944 spent 2 hrs and 40 min diving with only 26 minutes at the surface. Mean dive time was 6 min and 42 s ± 3 min 6 s while the mean surface interval was 1 min 6 s ± 1 min 39 s with the longest surface interval being 8 min and 35 s (46 s ± 27 s when 8 min 35 s surface interval is excluded from analysis). The longest dive duration for G5944 was 13 min and 14 s.

Seal G56882 had a similar dive pattern as compared with G5944 during the first 3 hrs of diving. There were 21 dives within the first 3 hours with a mean depth of 39.4 ± 12.9 m. Maximum depth for G5882 during the first 3 hours was 54.4 m. The mean dive duration was 7 min 45 s ± 3 min 26 s and mean surface time was 1 min 20 s ± 21 s. The maximum dive lasted 14 min and 15 s while the longest surface interval was 1 min and 45 s (Figure 9).

For the 77 dives from the oximeter logger for G5882, the mean depth was 146.14 ± 16.10 m with the shallowest dive at 2.56 m and the deepest dive (Dive 64) at 419.7 m (Figure 9). The seal spent a little over 16 hours diving and only a little over 2 hours at the surface. Mean dive duration was 12 min 31 s ± 6 min and 48 s. The minimum time spent diving was 15 s and the maximum dive was 25 minutes. The mean surface interval lasted 1 min 45 s ± 46 s with a minimum of 30 seconds and a maximum of 5 minutes.

All seals were equipped with GPS tags with ARGOS satellite uplink and had similar dive patterns to one another and to previous studies (Le Boeuf, Costa et al. 1986, Le Boeuf, Costa et al. 1986, Le Boeuf, Costa et al. 1988, Le Boeuf, Costa et al. 1988, Le Boeuf, Naito et al. 1989, Andrews, Jones et al. 1997, Le Boeuf, Crocker et al. 2000). Seals 6931, 6939, and 6841 did not have data from the oximeter logger, but all seals were equipped with the GPS/ARGOS loggers to give the seals positions and dive data. The GPS tracks for each animal are in Figure 7. All 5 seals had mean dive duration of between 13 and 16 minutes with a mean surface interval of 2-3 minutes. The mean surface interval included extended times at surface when the seals hauled back out at Pt. Lobos prior to crossing Monterey Bay.

Improvements to be made to sensor and data logger
Give our experience from the first field season the following improvements will be made:

1. **Battery:** The battery was located directly beneath the electronic board, which caused several problems. Wire placement makes replacing the battery difficult without damaging or pulling the wires. Also the battery was found to last only ~12-18 hours. Securing the battery within the housing is of utmost importance, as is extending the battery life.
2. **Internal Clock:** The previous logger used 3 LEDs and to save battery power the 940nm LED will be removed. Instead, an internal clock will be added to allow the data to be collected with a timestamp.
3. **Logger housing and sensor:** The logger housing and sensor head must be pressure tested to assure that pressure does not cause shift or "noise" in the data.
4. **On/Off switch:** The location and design of the magnetic on/off switch will be changed to allow accurate operation and prevent accidentally starting and stopping the data logger.

Aim 3:
This aim was completed in the last fiscal year. We collected tissues and determined the viability of enzymes for different methods to store the samples. We also determined myoglobin concentration in the muscle of elephant seals. These projects were published and used to for the thesis for a PhD.

Moore, C., Crocker, D. E., Fahlman, A., Moore, M., Willoughby, D. S., Robbins, K., Kanatous, S. B. and Trumble, S. J. (2014). Ontogenetic changes in skeletal muscle fiber type, fiber diameter and myoglobin concentration in the Northern elephant seal (Mirounga angustirostris). Frontiers in Physiology 5.

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IMPACT/APPLICATIONS

This project was intended to enhance our understanding of how the dive response alters muscle blood flow and metabolism in large, freely diving marine mammals. We completed the logger delivery device, confirmed that the absorbance spectra of marine mammal hemoglobin is similar to human, and showed that our sensor tip did not cause major trauma in elephants seals when implanted into the muscle. The MGH team has made major advances in developing a new type oximeter and have been working on validating this new unit. The knowledge gained from this worked has provided important knowledge for future work intended to generate a new generation of data loggers that are able to collect physiological data in large whales with minimal impact.

TRANSITIONS

None

RELATED PROJECTS

None

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